Five RecA-like Proteins of *Schizosaccharomyces pombe* Are Involved in Meiotic Recombination

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ABSTRACT

The genome of *Schizosaccharomyces pombe* contains five genes that code for proteins with sequence similarity to the *Escherichia coli* recombination protein RecA: *rad51*$^+$, *rhp55*$^+$, *rhp57*$^+$, *rlp1*$^+$, and *dmc1*$^+$. We analyzed the effect of deletion of each of these genes on meiotic recombination and viability of spores. Meiotic recombination levels were different from wild type in all *recA*-related mutants in several genetic intervals, suggesting that all five RecA homologs of *S. pombe* are required for normal levels of meiotic recombination. Spore viability was reduced in *rad51*, *rhp55*, and *rhp57* mutants, but not in *rlp1* and *dmc1*. It is argued that reduction of crossover is not the only cause for the observed reduction of spore viability. Analysis of double and triple mutants revealed that Rad51 and Dmc1 play major and partially overlapping roles in meiotic recombination, while Rhp55, Rhp57, and Rlp1 play accessory roles. Remarkably, deletion of Rlp1 decreases the frequency of intergenic recombination (crossovers), but increases intragenic recombination (gene conversion). On the basis of our results, we present a model for the involvement of five RecA-like proteins of *S. pombe* in meiotic recombination and discuss their respective roles.

HOMOLOGOUS recombination is an important process, which contributes to the genetic diversity within species and to the maintenance of genome stability. In bacteria, homologous recombination is promoted by RecA and other proteins. RecA forms a helical nucleoprotein filament on single-strand DNA (ssDNA), which searches for the homologous regions on double-strand DNA (dsDNA) and then displaces the homologous strand from the dsDNA and forms a heteroduplex with its complement. This activity of RecA is required for homologous recombination and recombinational repair of DNA double-strand breaks (DSB; for review, see Kowalczykowski and Eggleston 1994). Functions of RecA have been conserved in bacteriophage, prokaryotes, archaea, and eukaryotes. In eukaryotes RecA-like proteins have been found in a wide range of organisms from fungi to plants and mammals (for review see Shinohara and Ogawa 1999). In contrast to many bacteria that contain a single *recA* gene, eukaryotic genomes appear to contain multiple *recA*-like genes. There are four *recA*-like genes in *Saccharomyces cerevisiae*: *RAD51*, *RAD55*, *RAD57*, and the meiosis-specific *DMC1* (Aboussekhra *et al.* 1992; Basile *et al.* 1992; Bishop *et al.* 1992; Shinohara *et al.* 1992; Story *et al.* 1993; Donovan *et al.* 1994; Hays *et al.* 1995). In mammals, including humans, at least seven genes encoding proteins with considerable similarity to RecA/Rad51 have been identified.

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Budding yeast *rad51*, *rad55*, and *rad57* mutants have defects in homologous recombination and repair of DSBs in vegetative cells. Epistasis analysis suggests that all three genes act in the same pathway and that *RAD51* function is epistatic to those of *RAD55* and *RAD57* (Rattray and Symington 1995). Biochemical and genetical studies suggest that these RecA homologs do not have redundant functions, but rather have distinct roles to play in homologous recombination (for review see Paques and Haber 1999). Like RecA, *S. cerevisiae* Rad51 leads to the formation of nucleoprotein filaments on underwound DNA, the promotion of homologous pairing, and strand exchange reactions *in vitro* (Sung 1994; Baumann *et al.* 1996). In contrast to RecA, however, the budding yeast Rad51, like Rad51 from other eukaryotes, is considerably less efficient in these reactions, likely because it requires one or more accessory proteins. *S. cerevisiae* Rad55 and Rad57 form a heterodimer and do not seem to participate directly in strand exchange. Instead, they appear to act as a cofactor for the assembly of Rad51 onto ssDNA (Johnson and Symington 1995; Sung 1997). As there are several similarities between the Rad51 paralogs in yeast and in vertebrate cells, it is expected that some or all of the mammalian Rad51 paralogs might perform an analogous function. Physical interactions between Rad51 paralogs in budding yeast (and humans) have been demonstrated in yeast two- and three-hybrid systems and by co-immunoprecipitation (Hays *et al.* 1995; Johnson and Symington 1995; Tsutsui *et al.* 2001; Liu 2002; Lio *et al.* 2003). It has been proposed that during homologous recombination in mitosis, a recombinosome involving Rad51, Rad55,

Rad57, and several non-RecA-like proteins catalyzes the invasion of an unbroken DNA double helix by the 3′-ended single strand of the broken DNA (Hays *et al.* 1995; for review see Paques and Haber 1999).

Homologous recombination during yeast meiosis is initiated by DSBs and occurs at frequencies that are 100- to 1000-fold higher than those in vegetative cells. Normal formation of recombination products during meiosis in budding yeast requires *RAD51*, *RAD55*, and *RAD57* (Shinohara *et al.* 1992; Schwacha and Kleckner 1997). The additional *RAD51* paralog *DMC1* is strongly required for meiotic recombination and is expressed specifically in meiosis. Homologs of Dmc1 have been found in many other organisms (Terasawa *et al.* 1995; Habu *et al.* 1996; Doutriaux *et al.* 1998; Pittman *et al.* 1998) and are required in meiosis. In budding yeast *dmc1* mutants, meiotic recombination is strongly impaired, and completion of meiosis is prevented in some strains (Bishop *et al.* 1992; Rockmill *et al.* 1995; Dresser *et al.* 1997). Biochemical analysis of *S. cerevisiae* Dmc1 revealed that it shares several properties with RecA, suggesting that Dmc1 performs strand invasion (Hong *et al.* 2001). More advanced analysis of human Dmc1 revealed that it binds DNA and promotes strand transfer with homologous duplex DNA. However, in contrast to RecA and Rad51, hDmc1 did not form helical filaments on DNA. Instead, octameric rings were visualized on the DNA (Li *et al.* 1997; Passy *et al.* 1999). The relationship between Dmc1 and Rad51 in meiosis is unclear, with the main question being whether they act in one or two different pathways. Cytological analysis showed that at the time when meiotic recombination occurs in *S. cerevisiae* and mammals, Dmc1 and Rad51 form foci that co-localize to a high extent (Bishop 1994; Dresser *et al.* 1997; Tarsounas *et al.* 1999; Anderson *et al.* 2001). Epistatic analysis in budding yeast is complicated by the pachytene arrest, caused by *dmc1* mutation in some strains. A synergistic effect of *rad51* and *dmc1* has been observed on recombination in the SK1 genetic background (Shinohara *et al.* 1997), but this has been assessed in return-to-growth experiments, where the respective roles of mitotic and meiotic recombination pathways are unclear. Effects of *rad51* and *dmc1* mutations are different in different strain backgrounds (reviewed in Paques and Haber 1999).

In *Schizosaccharomyces pombe* five genes with homology to *recA* were identified: *rad51*$^+$, *rhp55*$^+$, *rhp57*$^+$, *rlp1*$^+$, and meiosis-specific *dmc1*$^+$ (Muris *et al.* 1993; Jang *et al.* 1994; Khasanov *et al.* 1999; Fukushima *et al.* 2000; Tsutsui *et al.* 2000; V. I. Bashkirov, personal communication). Rad51 binds ssDNA and dsDNA (Kim *et al.* 2002). As in *S. cerevisiae*, Rhp55 and Rhp57 form a stable heterodimer that interacts with Rad51 (Tsutsui *et al.* 2001). Similar to Rad51, Rad55, and Rad57 in *S. cerevisiae*, Rad51, Rhp55, and Rhp57 of *S. pombe* act in the same pathway for the repair of DSBs and the *rad51* mutant is more sensitive to DNA-damaging agents than are the *rhp55* and *rhp57* mutants (Khasanov *et al.* 1999; Tsutsui *et al.* 2000). Rad51 and Rhp57 are involved in homologous recombination in vegetative cells (Tsutsui *et al.* 2000).

During meiosis in fission yeast transcription of *dmc1*$^+$ is induced, and meiotic recombination in the *dmc1* mutant is reduced three- to fivefold (Fukushima *et al.* 2000; A. Grishchuk, R. Krähenbühl and M. Molnar, unpublished results). However, no arrest is observed during meiosis of the *dmc1* mutant, and the viability of spores is normal. Disruption of *rad51* has a much stronger effect on the viability of spores, compared to *dmc1*: survival of 2–8% of *rad51* deletion spores was reported (Muris *et al.* 1997; Khasanov *et al.* 1999; A. Grishchuk and M. Molnar, unpublished results). Muris *et al.* (1997) reported a 2.5-fold reduction in meiotic intergenic recombination in two genetic intervals tested in a *rad51* mutant. Intragenic recombination in a *rad51* mutant is reduced twofold (van den Bosch *et al.* 2002); Fukushima *et al.* (2000) did not measure the frequency of meiotic intragenic recombination because of poor spore formation and viability in *rad51* deletion. It has been claimed that Rad51 is required for repair of double-strand DNA breaks during fission yeast meiosis (Zenvirth and Simchen 2000). An *rhp55* null mutant was also reported to have a minor deficiency in meiotic intra- and intergenic recombination and spore viability (Khasanov *et al.* 1999).

All these data on the meiotic roles of Rad51 paralogs in *S. pombe* were obtained by different groups with different methods and markers for determination of recombination frequencies, making it difficult to compare the data. Not all *recA*-like genes were included in the analysis. Very limited epistasis analysis has been performed so far. A systematic analysis of the roles of Rad51 paralogs in meiosis and meiotic recombination is required to determine their respective roles.

It is of particular interest to perform this analysis in fission yeast. Genetic techniques are easily applicable to this unicellular eukaryote. Fission yeast has a fifth Rad51 paralog, Rlp1, that shows highest homology to the human XRCC2 and is absent from budding yeast (V. I. Bashkirov, personal communication). In *S. pombe* interaction of the Rhp55:Rhp57 heterodimer with Rad51 occurs via contact of Rad51 with Rhp57, while in *S. cerevisiae* Rad51 interacts with Rad55 (Tsutsui *et al.* 2001). In humans Rad51 interacts with its paralogs XRCC3 and Rad51C (Schild *et al.* 2000; Liu 2002), both of which have higher homology to Rhp57 than to Rhp55.

The aim of this study was to perform a systematic analysis of *S. pombe* RecA-like proteins to determine their respective roles during meiotic recombination and to analyze possible genetic interactions among these genes. Frequencies of gene conversions and crossovers were assayed by measuring the frequencies of intra- and intergenic recombination, respectively, in random spore

analysis. The contribution of non-Mendelian segregation to the formation of recombinants in crosses of markers in different genes (intergenic recombination) is negligible, since most gene mutations in fission yeast show a conversion frequency <1% (Gygax and Thuriaux 1984). Tetrad analysis performed on intragenic two-point crosses has revealed that intragenic crossovers are extremely rare (Gutz 1971; Gutz *et al.* 1974). Also, the viability of spores was determined. We report that all five RecA-like proteins of *S. pombe* play a role during meiosis. Rad51 and Dmc1 make unique contributions to meiosis; however, at least for some recombination events either one or both of them are needed. Rhp55, Rhp57, and Rlp1 have accessory functions. The observed reduction of crossover frequency in the mutant strains cannot explain the spore viability data obtained. We discuss the possible pathways of homologous recombination during meiosis in fission yeast and other functions of these genes contributing to spore viability.

MATERIALS AND METHODS

Strains, media, and growth conditions: The genotypes of *S. pombe* strains used in this study are listed in Table 1. The construction of *rad51*::*his3* and *dmc1*::*ura4* strains is described elsewhere (Grishchuk *et al.* 2003). The construction of the *rhp55*::*arg3* strain was described by Khasanov *et al.* (1999). The *rlp1*::*kanMX* strain was constructed by V. I. Bashkirov by replacement of the *rlp1* (SPBC1685.11) coding region with the *kanMX* marker, essentially as described by Bahler *et al.* (1998). This strain, as well as the *rhp55*::*arg3* strain, is a kind gift of V. I. Bashkirov. The *rhp57*::*ura4* strain is a kind gift of H. Shinagawa and its construction is described by Tsutsui *et al.* (2000). Standard *S. pombe* media yeast extract agar (YEA) and liquid (YEL), malt extract agar (MEA), and minimal medium (MMA) were described by Gutz *et al.* (1974). When necessary, 0.01% (w/v) of supplements was added to the media. Growing of *S. pombe* strains was performed at 30°. Crosses were performed at 25°.

Genetical methods and evaluation of crosses: In general the methods described by Gutz *et al.* (1974) were adopted. Crosses were set up as follows. Strains to be crossed were grown in YEL to $OD_{600} = 0.5$ (logarithmic phase). Equal amounts of cells ($\sim$3–8 $\times$ 10^7) of two parental strains were harvested, resuspended in a small volume of 0.85% NaCl (400 μl for each strain), mixed by vortexing, and plated on MEA plates. When large numbers of spores had to be analyzed (all multiple mutants), more cells were used for crosses. In this case, cells of each parental strain were resuspended to $\sim$1–3 $\times$ 10^8 cells/ml in 0.85% NaCl, mixed with a crossing partner by vortexing, and the suspension was evenly distributed on the entire surface of MEA plates (1 ml/plate). After 3 days the sporulated material was collected and treated overnight at 30° with snail digestive juice and lysing enzymes for digestion of vegetative cells and zygotes and dissolving of ascus walls. The spores were harvested and counted microscopically. For intergenic recombination analysis, the appropriately diluted spores were plated on YEA plates and grown to colonies at 30°. The spore colonies were randomly picked, grown on YEA master plates, and replica plated on MMA with appropriate supplements and MEA with h^+ and h^- tester strains to determine the numbers of recombinant (R) and parental (P) colonies. Spontaneously generated diploids were identified on the MEA plates and excluded from the quantification. Genetic distances in centimorgans were calculated as $d = -50 \ln(1 - 2[R/(R+P)])$. For intragenic recombination analysis the appropriately diluted spores were plated on YEA and MMA plates and grown to colonies at 30°. Prototrophic (recombinant) spore colonies were counted on selective media (MMA) and normalized to the number of viable spores determined by plating on nonselective rich media (YEA). Spore viability was determined in the crosses made to measure meiotic inter- and intragenic recombination as the ratio of colonies counted on the plates to the number of spores counted microscopically. A minimum of 200 spore colonies were counted in each independent experiment and the data from 6–15 independent experiments were used to calculate the mean spore viability and the standard error of the mean (SEM).

Calculation of frequency of spore death caused by missegregation: The mean numbers of chiasmata per bivalent and meiosis in fission yeast are 19, 15, and 11 for chromosomes I, II, and III, respectively (Munz 1994). For each mutant we counted the new number of chiasmata per each bivalent using the reduction of intergenic recombination, calculated as the mean of reductions in all intervals tested. For the new number of chiasmata per bivalent we calculated the probability that not a single chiasma is formed using the zero term of the Poisson distribution. Let this probability be x_1 for the pairs of chromosomes I, x_2 for II, and x_3 for III. Since we assumed that in the absence of chiasmata homologous chromosomes segregate randomly at MI, the probability of missegregation for each bivalent is $x_1/2$, $x_2/2$, and $x_3/2$, respectively, and the probability of correct segregation therefore is $1 - x_1/2$, $1 - x_2/2$, and $1 - x_3/2$. The probability that all three chromosome pairs segregate correctly during individual meiosis is $(1 - x_1/2)(1 - x_2/2)(1 - x_3/2)$. Since we assumed that the spores survive only in case of correct segregation of all three bivalents, the spore viability is expected to be $(1 - x_1/2)(1 - x_2/2)(1 - x_3/2)$.

RESULTS AND DISCUSSION

Intergenic recombination is reduced in all five *recA* mutants: To determine the contribution of the five Rad51 paralogs to meiotic recombination, and to be able to compare the results for different mutants directly, we measured recombination in parallel experiments in five isogenic strains, each deleted for a *recA*-like gene, using the same genetic intervals.

To study recombination events associated with crossovers, we measured the frequency of meiotic intergenic recombination in random spore analysis (see Introduction). Intergenic recombination was examined and the genetic distance in centimorgans was determined in three intervals on two chromosomes: *leu2-lys7* (right arm of chromosome I), *ade1-lys4*, and *ade1-mat1* (both on the right arm of chromosome II; Table 2). The results obtained for the three intervals were comparable, suggesting no regional specificity for the effect of the analyzed gene deletions on intergenic recombination. In contrast, some other *S. pombe* recombination mutants are known to have region-specific effects on recombination (DeVeaux and Smith 1994; Parisi *et al.* 1999). The strongest reduction was detected in *rad51* (5.6- to

TABLE 1

***S. pombe* strains used in this study**

Strain	Genotype
1-11	*h*$^+$ *ade1-40*
1-26	*h*$^-$ *ade7-50*
3-99	*h*$^-$ *lys4-95*
4-150	*h*$^+$ *lys7-2*
5-167	*h*$^-$ *ade6-M375*
6-216	*h*$^+$ *ade6-469*
AG55	*h*$^-$ *dmc1::ura4 leu2-120 ura4-D18*
AG56	*h*$^+$ *dmc1::ura4 lys7-2 ura4-D18*
AG121	*h*$^+$ *ade7-152*
AG124	*h*$^-$ *ade6-M375 dmc1::ura4 ura4-D18*
AG130	*h*$^+$ *ade6-469 dmc1::ura4 ura4-D18*
AG137	*h*$^-$ *ade7-50 dmc1::ura4 ura4-D18*
AG139	*h*$^-$ *leu2-120*
AG163	*h*$^+$ *ade7-152 dmc1::ura4 ura4-D18*
AG165	*h*$^+$ *ade7-152 his3-D1 rad51::his3*
AG167	*h*$^-$ *ade7-50 his3-D1 rad51::his3*
AG171	*h*$^+$ *ade7-152 arg3-D4 rhp55::arg3*
AG173	*h*$^-$ *ade7-50 arg3-D4 rhp55::arg3*
AG176	*h*$^+$ *ade6-469 his3-D1 rad51::his3*
AG178	*h*$^-$ *ade6-M375 his3-D1 rad51::his3*
AG181	*h*$^+$ *ade6-469 arg3-D4 rhp55::arg3*
AG183	*h*$^-$ *ade6-M375 arg3-D4 rhp55::arg3*
AG186	*h*$^-$ *his3-D1 leu2-120 rad51::his3*
AG190	*h*$^+$ *arg3-D4 lys7-2 rhp55::arg3*
AG192	*h*$^-$ *arg3-D4 leu2-120 rhp55::arg3*
AG195	*h*$^+$ *ade1-40 dmc1::ura4 ura4-D18*
AG196	*h*$^-$ *dmc1::ura4 lys4-95 ura4-D18*
AG198	*h*$^-$ *his3-D1 lys4-95 rad51::his3*
AG202	*h*$^+$ *ade1-40 rhp57::ura4 ura4-D18*
AG204	*h*$^-$ *lys4-95 rhp57::ura4 ura4-D18*
AG207	*h*$^+$ *ade1-40 arg3-D4 rhp55::arg3*
AG209	*h*$^-$ *arg3-D4 lys4-95 rhp55::arg3*
AG214	*h*$^+$ *ade1-40 his3-D1 rad51::his3*
AG216	*h*$^-$ *leu2-120 rhp57::ura4 ura4-D18*
AG220	*h*$^+$ *ade7-152 rhp57::ura4 ura4-D18*
AG222	*h*$^-$ *ade7-50 rhp57::ura4 ura4-D18*
AG226	*h*$^+$ *ade6-469 rhp57::ura4 ura4-D18*
AG228	*h*$^-$ *ade6-M375 rhp57::ura4 ura4-D18*
AG232	*h*$^+$ *lys7-2 rhp57::ura4 ura4-D18*
AG237	*h*$^-$ *lys4-95 rlp1::kanMX*
AG238	*h*$^+$ *lys7-2 rlp1::kanMX*
AG243	*h*$^-$ *leu2-120 rlp1::kanMX*
AG246	*h*$^+$ *ade1-40 rlp1::kanMX*
AG248	*h*$^+$ *ade7-152 rlp1::kanMX*
AG249	*h*$^-$ *ade7-50 rlp1::kanMX*
AG252	*h*$^+$ *ade6-469 rlp1::kanMX*
AG253	*h*$^-$ *ade6-M375 rlp1::kanMX*
AG256	*h*$^+$ *his3-D1 lys7-2 rad51::his3*
AG337	*h*$^-$ *dmc1::ura4 his3-D1 lys4-95 rad51::his3 ura4-D18*
AG340	*h*$^+$ *ade1-40 dmc1::ura4 his3-D1 rad51::his3 ura4-D18*
AG341	*h*$^-$ *ade7-50 dmc1::ura4 his3-D1 rad51::his3 ura4-D18*
AG344	*h*$^+$ *ade7-152 dmc1::ura4 his3-D1 rad51::his3 ura4-D18*
AG403	*h*$^-$ *dmc1::ura4 lys4-95 rlp1::kanMX ura4-D18*

(*continued*)

TABLE 1

(Continued)

Strain	Genotype
AG408	*h*$^+$ *ade1-40 dmc1::ura4 rlp1::kanMX ura4-D18*
AG409	*h*$^-$ *ade7-50 dmc1::ura4 rlp1::kanMX ura4-D18*
AG412	*h*$^+$ *ade7-152 dmc1::ura4 rlp1::kanMX ura4-D18*
AG418	*h*$^+$ *ade1-40 his3-D1 rlp1::kanMX rad51::his3*
AG419	*h*$^-$ *ade7-50 his3-D1 rlp1::kanMX rad51::his3*
AG422	*h*$^+$ *ade7-152 his3-D1 rlp1::kanMX rad51::his3*
AG425	*h*$^-$ *his3-D1 lys4-95 rlp1::kanMX rad51::his3*
AG434	*h*$^-$ *arg3-D4 his3-D1 lys4-95 rad51::his3 rhp55::arg3 rhp57::ura4 ura4-D18*
AG437	*h*$^+$ *ade1-40 arg3-D4 his3-D1 rad51:: his3 rhp55::arg3 rhp57::ura4 ura4-D18*
AG438	*h*$^-$ *ade7-50 arg3-D4 his3-D1 rad51::his3 rhp55::arg3 rhp57::ura4 ura4-D18*
AG441	*h*$^+$ *ade7-152 arg3-D4 his3-D1 rad51::his3 rhp55::arg3 rhp57::ura4 ura4-D18*
AG442	*h*$^-$ *arg3-D4 lys4-95 rhp55::arg3 rhp57::ura4 ura4-D18*
AG443	*h*$^+$ *ade1-40 arg3-D4 rhp55::arg3 rhp57::ura4 ura4-D18*
AG445	*h*$^-$ *ade7-50 arg3-D4 rhp55::arg3 rhp57::ura4 ura4-D18*
AG448	*h*$^+$ *ade7-152 arg3-D4 rhp55::arg3 rhp57::ura4 ura4-D18*
AG449	*h*$^-$ *arg3-D4 his3-D1 lys4-95 rad51::his3 rhp55::arg3*
AG452	*h*$^+$ *ade1-40 arg3-D4 his3-D1 rad51::his3 rhp55::arg3*
AG453	*h*$^-$ *ade7-50 arg3-D4 his3-D1 rad51::his3 rhp55::arg3*
AG456	*h*$^+$ *ade7-152 arg3-D4 his3-D1 rad51::his3 rhp55::arg3*
AG459	*h*$^-$ *his3-D1 lys4-95 rad51::his3 rhp57::ura4 ura4-D18*
AG462	*h*$^+$ *ade1-40 his3-D1 rad51::his3 rhp57::ura4 ura4-D18*
AG463	*h*$^-$ *ade7-50 his3-D1 rad51::his3 rhp57::ura4 ura4-D18*
AG465	*h*$^+$ *ade7-152 his3-D1 rad51::his3 rhp57::ura4 ura4-D18*
AG466	*h*$^-$ *arg3-D4 dmc1::ura4 lys4-95 rhp55::arg3 rhp57::ura4 ura4-D18*
AG469	*h*$^+$ *ade1-40 arg3-D4 dmc1::ura4 rhp55::arg3 rhp57::ura4 ura4-D18*
AG470	*h*$^-$ *ade7-50 arg3-D4 dmc1::ura4 rhp55::arg3 rhp57::ura4 ura4-D18*
AG473	*h*$^+$ *ade7-152 arg3-D4 dmc1::ura4 rhp55::arg3 rhp57::ura4 ura4-D18*

All strains marked with AG were constructed for this study. All other strains were from the strain collection in Bern.

TABLE 2

Intergenic recombination

	Strains crossed (genetic interval)[a]	Recombinant frequency RF = $R/(R + P)$; mean ± SEM[b]	Range of RF values from individual experiments (no. of experiments)	Genetic distance (cM): $-50\ln(1 - 2[R/(R+P)])$; mean ± SEM[b]	Average reduction (fold)
Wild type	3-99 × 1-11 (A)	27 ± 1.3	25.5–29.5 (3)	38.8 ± 1.3	1
	3-99 × 1-11 (B)	43 ± 2.1	40–47 (3)	98.3 ± 2.1	1
	AG139 × 4-150 (C)	11.7 ± 1.1	9–14.2 (4)	13.3 ± 1.1	1
dmc1	AG196 × AG195 (A)	7 ± 0.8	5.1–9 (4)	7.6 ± 0.8	5.1
	AG196 × AG195 (B)	18.8 ± 2.8	16–27 (4)	23.6 ± 2.9	4.2
	AG55 × AG56 (C)	2.9 ± 0.6	1.3–4.5 (5)	3.0 ± 0.6	4.5
rad51	AG198 × AG214 (A)	6.5 ± 0.8	5–8.7 (4)	6.9 ± 0.8	5.6
	AG198 × AG214 (B)	10.9 ± 1.7	7.8–15 (4)	12.3 ± 1.8	8
	AG186 × AG256 (C)	2.1 ± 0.3	1.6–2.7 (3)	2.1 ± 0.3	6.3
rhp55	AG207 × AG207 (A)	13 ± 0.5	12–13.5 (3)	15 ± 0.5	2.6
	AG207 × AG207 (B)	28.1 ± 1	26.5–30 (3)	41.3 ± 1	2.5
	AG192 × AG190 (C)	4.4 ± 0.5	2.8–5.1 (4)	4.6 ± 0.5	2.6
rhp57	AG204 × AG202 (A)	13 ± 1.3	10–16 (5)	15.1 ± 1.3	2.6
	AG204 × AG202 (B)	27.2 ± 2.3	19–33 (5)	39.4 ± 2.4	2.4
	AG216 × AG232 (C)	4.8 ± 0.1	4.7–5 (3)	5.1 ± 0.1	2.9
rlp1	AG237 × AG246 (A)	12.5 ± 0.8	11.5–14.1 (3)	14.4 ± 0.8	2.7
	AG237 × AG246 (B)	32.5 ± 1.5	31–35.5 (3)	52.5 ± 1.5	1.9
	AG243 × AG238 (C)	7.7 ± 0.7	5.9–8.8 (4)	8.3 ± 0.7	1.6
rad51 dmc1	AG337 × AG340 (A)	0.1 ± 0.04	0; 0.1; 0.14; 0.21[c]	0.1 ± 0.04	345
	AG337 × AG340 (B)	0.1 ± 0.04	0; 0.1; 0.14; 0.21[c]	0.1 ± 0.04	873
rlp1 dmc1	AG403 × AG408 (A)	5.7 ± 0.6	4.1–6.6 (4)	6.1 ± 0.6	6.4
	AG403 × AG408 (B)	16.4 ± 0.9	14.6–18.9 (4)	19.8 ± 0.9	5
rlp1 rad51	AG425 × AG418 (A)	6.8 ± 0.5	5.8–7.6 (4)	7.3 ± 0.5	5.3
	AG425 × AG418 (B)	14.9 ± 1	12.8–17.5 (4)	17.7 ± 1	5.5
rhp55 rhp57	AG434 × AG437 (A)	12.1 ± 0.9	10.4–13.4 (3)	13.9 ± 0.9	2.8
	AG434 × AG437 (B)	27.9 ± 2	25.5–32 (3)	40.9 ± 2.1	2.4
rad51 rhp55 rhp57	AG442 × AG443 (A)	6.4 ± 0.7	5.0–7.2 (3)	6.8 ± 0.7	5.7
	AG442 × AG443 (B)	15.9 ± 0.7	14.8–17.2 (3)	19.1 ± 0.7	5.1
rad51 rhp55	AG449 × AG452 (A)	7.1 ± 0.5	6.2–8 (3)	7.7 ± 0.5	5
	AG449 × AG452 (B)	14.2 ± 1.6	11.1–16.6 (3)	16.7 ± 1.6	5.9
rad51 rhp57	AG459 × AG462 (A)	5.5 ± 0.2	5.1–5.9 (3)	5.8 ± 0.2	6.7
	AG459 × AG462 (B)	15.7 ± 1	14.2–17.7 (3)	18.8 ± 1.1	5.2
dmc1 rhp55 rhp57	AG466 × AG469 (A)	3.7 ± 0.5	2.6–4.2 (3)	3.8 ± 0.5	10.2
	AG466 × AG469 (B)	8.7 ± 2.5	3.7–11.1 (3)	9.6 ± 2.6	10.2

A minimum of 200 colonies were analyzed in each independent experiment.

[a] Genetic intervals: (A) *ade1-lys4*; (B) *ade1-mat1*; (C) *leu2-lys7*.

[b] R is the number of recombinant colonies; P is the number of parental colonies.

[c] Because the values were small, they were all given instead of the range.

8.0-fold) and *dmc1* (4.2- to 5.1-fold) knockouts (Table 2). The difference between these two mutants is not significant in two cases, and standard errors of the means are almost overlapping in the third case. *rhp55*, *rhp57*, and *rlp1* mutants show moderate reductions of genetic distance (2.4- to 2.9-, 2.5- to 2.6-, and 1.6- to 2.7-fold, respectively). *rhp55* and *rhp57* showed similar mean reductions in all intervals tested, as was expected since Rhp55 and Rhp57 form a complex supporting Rad51 in mitotic DNA repair (Tsutsui *et al.* 2001). Our data are not in contradiction with previously reported results.

Intragenic recombination is reduced in all mutants except *rlp1*: To study gene conversion, we measured the frequency of meiotic intragenic recombination in random spore analysis (see Introduction). The frequency of formation of wild-type (prototrophic) recombinants was examined in the *ade7* locus on the left arm of chromosome II between *ade7-50* and *ade7-152* and in the *ade6* locus on the right arm of chromosome III between *ade6-469* and *ade6-M375*. The results obtained for both intervals were comparable, suggesting no region specificity for the effect of the gene deletions on intragenic recombination (Table 3). The strongest reduction in prototroph frequency was detected in the *rad51* mutant (17.1- to 25.2-fold). The reduction in the *dmc1* mutant (2- to 2.3-fold) was much weaker than

TABLE 3

Intragenic recombination

	Strains crossed (genetic interval)[a]	Prototroph frequencies ade^+/total $\times 10^6$; mean ± SEM	Range of prototroph frequencies in individual experiments (no. of experiments)	No. of viable spores analyzed in individual experiments[b]	Average reduction (fold)
Wild type	AG161 × AG162 (D)	500 ± 37	407–590 (4)		1
	5-167 × 6-216 (E)	363 ± 34	231–446 (6)		1
dmc1	AG137 × AG163 (D)	245 ± 26	207–296 (3)		2
	AG124 × AG130 (E)	158 ± 5	146–169 (4)		2.3
rad51	AG167 × AG165 (D)	19.8 ± 7.1	0; 4; 8; 11; 29; 42; 46[c]	2.5×10^6	25
	AG178 × AG176 (E)	21.2 ± 7.3	0; 11; 22; 34, 39[c]	5.2×10^6	17
rhp55	AG173 × AG171 (D)	424 ± 63	135–715 (8)		1.3
	AG183 × AG181 (E)	184 ± 33	75–323 (6)		1.9
rhp57	AG222 × AG220 (D)	393 ± 17	367–424 (3)		1.2
	AG228 × AG226 (E)	188 ± 6.5	176–198 (3)		2.0
rlp1	AG249 × AG248 (D)	677 ± 63	434–863 (6)		0.7
	AG253 × AG252 (E)	503 ± 55	277–639 (6)		0.7
rad51 dmc1	AG341 × AG344 (D)	<0.46[d]	0 (5)	2.2×10^5–4.3×10^6	>1000
rlp1 dmc1	AG409 × AG412 (D)	591 ± 33	519–674 (4)		0.8
rlp1 rad51	AG419 × AG422 (D)	148 ± 23	84–185 (4)		3.4
rhp55 rhp57	AG445 × AG448 (D)	245 ± 21	198–273 (3)		2
rad51 rhp55 rhp57	AG438 × AG441 (D)	79.8 ± 9.5	64–105 (3)		6.3
rad51 rhp55	AG453 × AG456 (D)	38.8 ± 14	0; 38; 58; 59[c]	2.6×10^8	13
rad51 rhp57	AG463 × AG465 (D)	86.7 ± 4.6	79–95 (3)		5.8
dmc1 rhp55 rhp57	AG470 × AG473 (D)	9.3 ± 9.3	0; 0; 28[c]	4.2×10^6; 1.6×10^7	54

A minimum of 150 prototroph colonies were counted in each independent experiment except the following cases: *rad51* (D) (0, 3, 79, 95, 232, 699, 1091, 1850), *rad51* (E) (0, 58, 70, 202, 1195), *rad51 dmc1* (0, 0, 0, 0, 0), *rad51 rhp55* (0, 1402, 1717, 3533), and *dmc1 rhp55 rhp57* (0, 0, 514).

[a] Genetic intervals: (D) *ade7-50-ade7-152*; (E) *ade6-469-ade6-M375*.

[b] This number is given for those experiments where no prototrophic colonies were detected.

[c] Because the values were small, they were all given instead of the range.

[d] No prototrophs were detected among a total of 6.5×10^6 viable spores plated. The number of prototrophs was set to three, which resulted in the calculated prototroph frequency of 0.46 per 10^6 viable spores. The true frequency is 0.46 per 10^6 or less with 95% certainty according to the Poisson distribution, or 0.1% or less relative to wild type.

that in *rad51*. *rhp55* and *rhp57* mutants showed similar defects, although they were not always significantly different from wild type. The *rlp1* mutant had an insignificant hyperrecombination effect, which, however, became clearly apparent by analysis of double mutants (see below).

Taken together, the data on meiotic inter- and intragenic recombination reveal that all five gene products are involved in meiotic recombination, as meiotic recombination levels are different from wild type in all mutants in at least three or more of the tested intervals.

Spore viability in *rad51*, *rhp55*, and *rhp57* mutants is reduced: The reduction of meiotic recombination, in particular intergenic, can result in decrease of viability of spores: in the absence of chiasmata, results of crossovers, homologs missegregate more frequently during meiosis I (MI; Molnar *et al.* 2001). We detected no significant reduction in viability of spores in *dmc1* and *rlp1* mutants, while *rad51*, *rhp55*, and *rhp57* mutants showed reduced survival of spores (Figure 1). To estimate if the observed reduction in intergenic recombination frequency could cause the detected reduction in spore viability, we estimated the probability of missegregation of homologs in the analyzed mutants and compared these data with the detected spore viability. Several assumptions were made to simplify the calculations. First, we assumed that in the absence of chiasmata homologs segregate randomly at MI. Second, we assumed that missegregation of homologs does not preclude spore formation. Finally, we assumed that during an individual meiosis missegregation of at least one homologous pair in MI results in formation of four inviable spores. We disregarded the fact that disomes for chromosome III are viable (Niwa and Yanagida 1985) and that even in the absence of chiasmata homologs segregate nonrandomly (Molnar *et al.* 2001; Davis and Smith 2003); therefore, the calculated viability is an underestimate, and the real value is likely to be higher. See materials and methods for the details of calculation. The strongest reduction of intergenic recombination was observed in the *rad51* mutant. This reduction, according to our estimation, would result in a spore

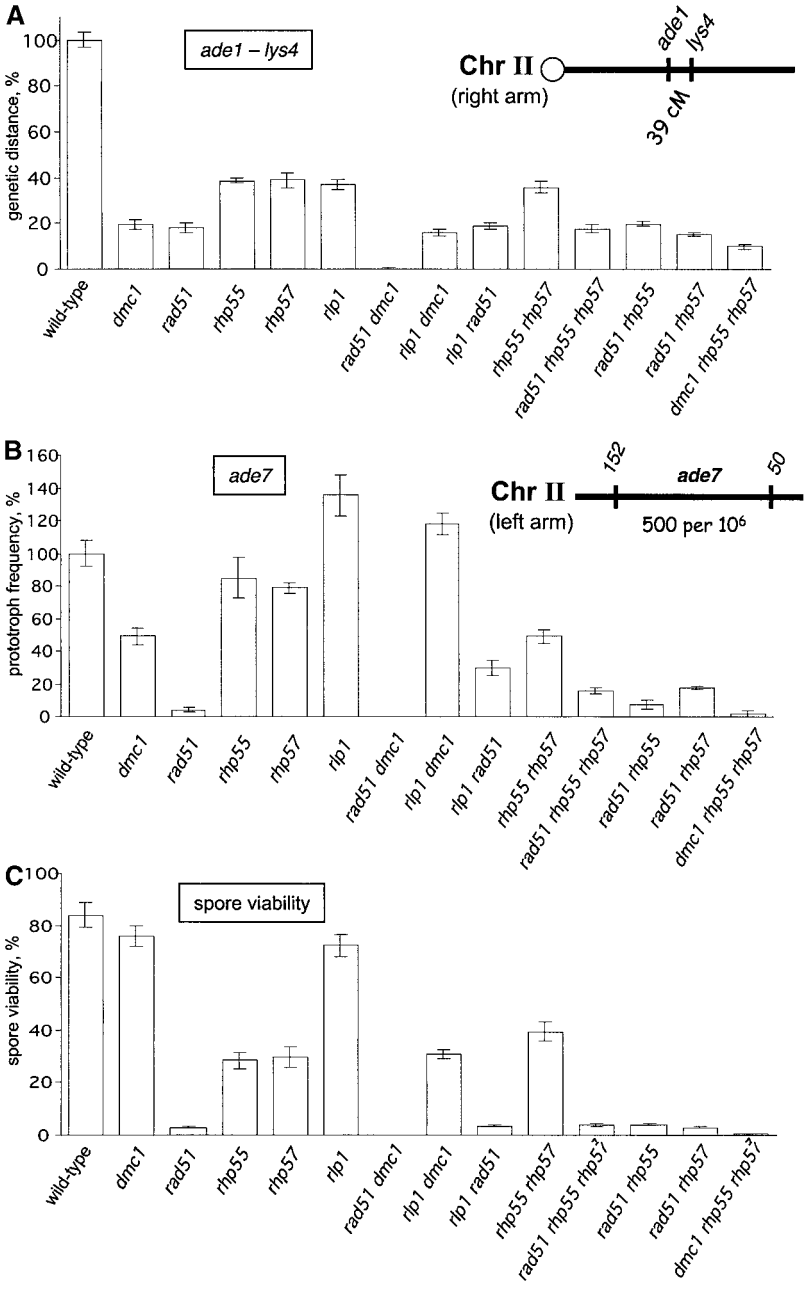

FIGURE 1.—Meiotic recombination and spore viability in single- and multiple-*recA* mutants. The genes indicated under the bars are those that were deleted. (A) Intergenic recombination. Genetic distances are given relative to wild type (100%). The diagram on the right schematically shows the position of markers used to measure intergenic recombination and the distance between them in centimorgans calculated for wild type. The centromere is shown as an open circle. (B) Intragenic recombination. The prototrophic recombinant frequencies are given relative to wild type (100%). Because prototroph frequency is calculated as a fraction of surviving cells in each cross, the sensitivity of detection of recombinants decreases as the viability decreases. The diagram on the right schematically shows the markers used to measure intragenic recombination and the number of prototrophic recombinants determined per 10^6 viable spores for wild type. (C) Spore viability.

viability of 76.9%. The spore viability of *rhp55*, *rhp57*, and *rlp1* was expected to be >98% and that of dmc1 >90%. The observed spore viability in *rad51*, *rhp55*, and *rhp57* mutants (2.9 ± 0.3%, 28.5 ± 3%, and 29.8 ± 4.1%, respectively) was lower than predicted. Thus, the calculated missegregation of homologs due to reduced number of chiasmata cannot account for the observed reduction of spore viability. For comparison, a completely random segregation (in the absence of recombination) should result in survival of 12.5% of spores according to our calculations; thus the low viability observed in the *rad51* mutant cannot be caused simply by faulty segregation due to the reduction of recombination. Thirty percent viability (like that in the *rhp55* and *rhp57* mutants) could be expected if intergenic recombination is reduced ~35-fold, which is >10 times more than that observed in these mutants.

Along with contributing to correct chromosome segregation through meiotic recombination, we see additional ways that Rad51, Rhp55, and Rhp57 can influence the survival of spores. Meiotic recombination in *S. pombe* is initiated by DSBs (CERVANTES *et al.* 2000). In addition, breaks and stalled replication forks arise during bacterial and premeiotic DNA replication (MICHEL *et al.* 1997; SOGO *et al.* 2002). Since *rad51*, *rhp55*, and *rhp57* mutants show mitotic slow growth (JANG *et al.* 1995; KHASANOV *et al.* 1999; GRISHCHUK *et al.* 2003), they may also suffer damage in meiotic DNA replication and afterward.

These may not be DSBs at all. Programmed DSBs as well as DNA damage occurring independently of meiotically programmed DSBs must be repaired to allow viable progeny. Rad51, Rhp55, and Rhp57 are required for repair of programmed DSBs, with Rad51 playing a major role. But the repair of DNA damage other than DSBs by Rad51, Rhp55, and Rhp57 could also contribute to spore viability.

Because spore viability requires proficient recombinational repair, the dead spores may actually have had less recombination events than the surviving ones. This means that the recombination level detected by the genetical assays (especially in the severe recombination mutants) may actually be an overestimate, since only surviving spores are considered. Detection of the recombination events at the DNA level (physical recombination assays) may give a better estimate of recombination levels in mutants and is planned as a follow-up experiment.

Rad51 and Dmc1 play major and partially overlapping roles in meiotic recombination: Analysis of double and multiple mutants allows the assignment of genes to a single or to different epistasis groups. To determine epistasis relationship among the five *S. pombe* Rad51 paralogs, we assayed double and triple mutants for the same phenotypes as the single mutants: spore viability and meiotic inter- and intragenic recombination frequencies. Taking into account the similarity of results obtained for recombination frequencies in different intervals for single mutants, we limited the analysis of multiple mutants to two intergenic (*ade1-lys4*, *ade1-mat1*) and one intragenic interval (*ade6* locus). The *dmc1 rad51* double mutant produced markedly fewer viable spores than did either single mutant: $0.2 \pm 0.05\%$ (Figure 1). The true value may even be $<0.2\%$, because cells that survived after the snail enzyme treatment (see MATERIALS AND METHODS) may also form colonies. The contribution of this factor is the greater, as the viability of spores decreases. Low sporulation efficiency ($<9\%$, data not shown) and extremely low viability of the double mutant make it too difficult to analyze large numbers of spores. Genetic distance between the analyzed markers decreased dramatically in the *dmc1 rad51* double mutant compared to that in either single mutant (Table 2, Figure 1). Because intergenic recombination frequency is calculated as a fraction of colony-forming units, our value may be an underestimate. However, two of the four observed recombinants had the genotype characteristic for a double crossover, which is very unlikely to occur in this cross with dramatically reduced recombination frequency. One of these recombinants, and an additional one of the remaining two, grew much more slowly than expected. We assume that these recombinants arose not by a regular meiotic recombination event, but probably by some rare irregular mechanism involving chromosome rearrangement. Analysis of intragenic recombination in the *dmc1 rad51* double mutant revealed no prototrophic recombinant progeny among 6.5×10^6 viable spores analyzed. In wild type this number of viable spores would contain ~3000 recombinants. The extremely low spore viability and almost undetectable meiotic inter- and intragenic recombination suggest that the remaining Rad51 paralogs are not able to carry out recombination and repair on their own. We therefore conclude that Rad51 and Dmc1 play major roles in meiotic recombination and survival of spores, while Rhp55, Rhp57, and Rlp1 play accessory roles. This conclusion is in agreement with the finding of J. Mata *et al.* who showed in the microarray experiment that the transcription of *rad51*$^+$ and *dmc1*$^+$ is significantly induced during meiotic prophase, leading to higher amounts of these two mRNAs compared to other *recA*-like genes (MATA *et al.* 2002; J. MATA, personal communication).

Budding-yeast *DMC1* and *RAD51* do not show a simple epistasis relationship. They were proposed to have distinct and overlapping roles in meiotic recombination (DRESSER *et al.* 1997; SHINOHARA *et al.* 1997). The results presented in this work, together with findings of other groups (ZENVIRTH and SIMCHEN 2000; SHIMADA *et al.* 2002), provide evidence that *S. pombe* Rad51 and Dmc1 also play distinct and overlapping roles.

Distinct roles for Rad51 and Dmc1: There is increasing evidence that *S. cerevisiae* cells have separate pathways leading to crossover and noncrossover recombinants, both of which are initiated by DSBs and are associated with new DNA synthesis and formation of heteroduplex DNA, and thus may lead to gene conversion (ALLERS and LICHTEN 2001; HUNTER and KLECKNER 2001). We found that intragenic recombination in the *rad51* mutant was reduced much more than that in the *dmc1* mutant, in contrast to intergenic recombination. This could indicate that Rad51 is more important than Dmc1 for conversions, but at the same time Dmc1 and Rad51 are equally important for recombination events associated with crossovers. These results are not easily explained by a single recombination pathway starting with gene conversion and leading to resolution both with crossover and without crossover. In contrast, our observations are consistent with the existence of more than one pathway for meiotic recombination, for example, one pathway for gene conversion associated with crossing over and another one for gene conversion that cannot lead to crossing over. Rad51 and Dmc1 may be of different importance for these pathways. In addition, deletions of Rad51 and Dmc1 have different effects on spore viability. In brief, our data suggest (i) that Dmc1 and Rad51 are of different importance for pathways of meiotic recombination leading to crossovers and noncrossovers and (ii) that Rad51 has a larger effect on the viability of spores through its involvement in repair of DNA damage other than the DSBs caused by Rec12 (SPO11 homolog) for initiation of recombination. Thus, Rad51 and Dmc1 appear to play distinct roles

during meiosis. Additional evidence supporting these interpretations has been published. Meiotic DSBs in *rad51* cells do not disappear at late points (6 hr after initiation of meiosis; Zenvirth and Simchen 2000), while in *dmc1* cells DSBs are diminished at the same time (4.5 hr) as they are diminished in the control (*dmc1*$^+$) cells (Shimada *et al.* 2002).

Overlapping roles for Rad51 and Dmc1: The elimination of both proteins confers a greater effect on the viability of spores and on meiotic recombination than does the sum of eliminations of either protein alone (Figure 1, Tables 2 and 3). Thus, it seems probable that Rad51 and Dmc1 have an overlapping capacity to catalyze one or more of the steps of recombination. As was proposed for *S. cerevisiae* Rad51 and Dmc1 (Shinohara *et al.* 1997), this redundant functional capacity could be one that is shared with RecA.

Rhp55 and Rhp57 act in the same pathway with Rad51 and stimulate its activity: Our experiments revealed that in meiosis the *rhp55 rhp57* double mutant is very similar to either single mutant with respect to spore viability and intergenic recombination (Figure 1, Tables 2 and 3). This observation suggests that the two proteins function in the same meiotic pathways. Intragenic recombination is slightly lower in the double mutant than in the single mutants, and spore viability is slightly higher. We have no explanation for these phenomena. It may be that the difference between the mutants is not biologically significant, even though it is mathematically significant. A small difference between the double and single mutants was observed in *S. cerevisiae* with respect to γ-ray sensitivity (Johnson and Symington 1995): a *rad55 rad57* double mutant was found to be slightly more sensitive to γ-irradiation at 37° than either of the single mutants.

The double mutants *rad51 rhp55*, *rad51 rhp57* as well as the triple mutant *rad51 rhp55 rhp57* show the same reduction in spore viability and intergenic recombination as the *rad51* single mutant. These results suggest that *rad51* is epistatic to both *rhp55* and *rhp57* in meiosis. This indicates that all three proteins act in the same pathway with respect to meiotic recombination and spore viability.

The spore viability and intragenic recombination levels are much higher in the presence of Rad51 together with Rhp55, Rhp57, and Rlp1 (*dmc1* single mutant) than in the presence of only Rad51 and Rlp1 (*dmc1 rhp55 rhp57* triple mutant). This effect is much less pronounced, but still significant, in intergenic recombination. Taking into account the finding that Rad51, Rhp55, and Rhp57 act in the same pathway, we suggest that Rhp55 and Rhp57 stimulate the activity of Rad51.

These observations together with the previously published investigations of Rad51, Rhp55, and Rhp57 (Khasanov *et al.* 1999; Tsutsui *et al.* 2000) reveal the similarity between *S. pombe* and *S. cerevisiae*, where Rad51/55/57 proteins also work intimately together and Rad55/57 stimulate the activity of Rad51. However, there are also some differences concerning this complex between the two yeasts. One concerns the nature of interaction between Rad51 and the Rhp55/57 heterodimer and was mentioned in the Introduction. Another is discussed in the following paragraph.

Dmc1 is partially inhibited by Rhp57 and probably also by Rhp55: Intragenic recombination in the *rad51* mutant (contains intact *dmc1*$^+$, *rlp1*$^+$, and *rhp55*$^+$/*57*$^+$ genes) was reduced 25-fold (Figure 1, Table 2), while in the *rad51 rhp55 rhp57* triple mutant (contains intact *dmc1*$^+$ and *rlp1*$^+$ genes) it was reduced only 6.3-fold. This suggests that in the *presence* of Rhp55/57 intragenic recombination carried out by Dmc1 (in the absence of Rad51) is *less* efficient. To our knowledge no genetic interaction between *S. cerevisiae* Dmc1 and Rad55 or Rad57 has been reported. Intragenic recombination in the *rad51 rhp57* mutant is reduced to the same range as in the *rad51 rhp55 rhp57* mutant (5.8-fold), while in *rad51 rhp55* it is reduced to the range of the *rad51* mutant (13-fold). If the difference between the mutants is not only statistically but also biologically significant, it may indicate that Rhp57 rather than Rhp55 partially inhibits recombination carried out by Dmc1. To our knowledge no genetic interaction between *S. cerevisiae* Dmc1 and Rad55 or Rad57 has been reported.

Deletion of *rlp1*$^+$ has different effects on meiotic inter- and intragenic recombination and spore viability: The double mutant *rlp1 dmc1* shows lower spore viability (30.9 ± 1.7%) compared to either single mutant (*rlp1*, 72.4 ± 4.3%; *dmc1*, 75.9 ± 3.8%), while the spore viability in the *rlp1 rad51* double mutant (3.5 ± 0.4%) is as low as in *rad51* alone (2.9 ± 0.3%). This indicates that with respect to spore viability *rlp1* is in the same epistasis group with *rad51* and in a different one than *dmc1*.

Intergenic recombination in the *rlp1 rad51* and *rlp1 dmc1* double mutants is lower than that in the *rlp1* single mutant and similar to that in the the *rad51* and *dmc1* single mutants (Figure 1, Table 2). Thus, with respect to intergenic recombination *rlp1* is in the same epistasis group as *rad51* and also as *dmc1*. But *rad51* and *dmc1* are in different epistasis groups with respect to intergenic recombination (see above). Since the levels of intergenic recombination in *rad51* and *dmc1* mutants are similar, and the same is observed for the *rlp1 rad51* and *rlp1 dmc1* double mutants, it is not clear how the Rlp1 protein contributes to crossover formation in the Dmc1- and Rad51-directed pathways.

Deletion of the *rlp1*$^+$ gene increases intragenic recombination frequencies in both *rad51* and *dmc1* mutants, as can be seen from the comparison of single and double mutants (Figure 1, Table 3). Reduction of intragenic recombination in the *rad51* mutant is more pronounced than that in the *dmc1* mutant, and the same is observed for *rlp1 rad51* and *rlp1 dmc1* double mutants. This indicates that *rlp1*$^+$ has a role in intragenic recombination events dependent on *rad51*$^+$, as well as in those depen-

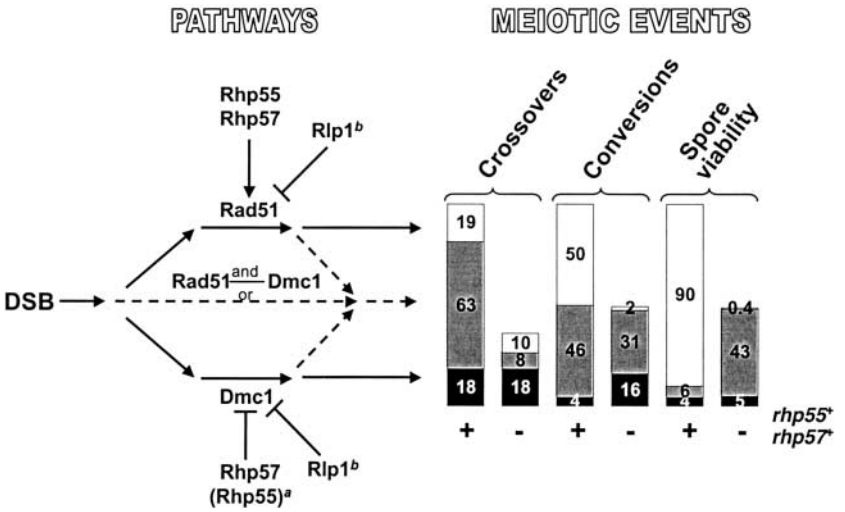

FIGURE 2.—A model of the contribution of *S. pombe* RecA-like proteins to different meiotic events. The respective contributions of three tentative pathways in presence and absence of Rhp55/Rhp57 (*rlp1*$^+$ is always present) are indicated with shadings and numbers (in percentage of the total observed in wild type). Presence and absence of Rhp55 and Rhp57 are indicated with + and −, respectively. See the text for more information. *a*, the inhibitory effect of Rhp57 (and probably Rhp55) on Dmc1 is observed only in intragenic recombination (conversion). *b*, the inhibitory effect of Rlp1 on Rad51 and Dmc1 is observed only in intragenic recombination (conversion).

dent on *dmc1*$^+$. On the basis of data presented, it is difficult to explain the differences in the genetic interaction of *rlp1*$^+$ with *rad51*$^+$ and *dmc1*$^+$ in different assays. However, it is important to keep in mind that the viability of spores not only depends on the level of meiotic recombination but also may be influenced by additional, not yet known, roles of *rlp1*$^+$ and its interactions with *rad51*$^+$ and *dmc1*$^+$, both of which also were shown to have differential effects on meiotic recombination and viability of spores (see above).

We have developed two hypotheses to explain the hyperrecombination phenotype of *rlp1* with respect to gene conversion. First, Rlp1 may be involved in the regulation of the length of conversion tracts during recombination. If the DSB occurs outside the interval between the two markers used for measurement of intragenic recombination, and Rlp1 is required to extend the conversion tract, deletion of *rlp1*$^+$ would lead to shortening of heteroduplex length and consequently to an increase of independent conversion of the two markers, resulting in an increase of prototrophic recombinants. But Rlp1 may actually limit hybrid DNA extension starting from the DSB, instead of increasing it. Deletion of *rlp1*$^+$ would then lead to an increase of hybrid DNA length and the marker closer to the DSB may be converted more frequently than in *rlp1*$^+$ cells to produce prototrophic recombinants. Obviously, this lengthening of hybrid DNA should not lead to more frequent coverage of the second marker: coconversion of both markers would reduce prototroph frequency. To test whether Rlp1 influences the length of hybrid DNA tracts (or other parameters affecting length of conversion tracts), tetrads of two-factor crosses have to be analyzed to estimate the amounts of single-site and coconversion events. Also, the position of the DSB site relative to the markers has to be determined.

Alternatively, Rlp1 may be involved in promoting conversion between sister chromatids. In this case a shift from undetectable sister chromatid conversion to detectable homolog conversion may happen in the *rlp1* mutant, accounting for the higher frequency of intragenic recombination in this mutant.

Working hypothesis on roles of RecA-like proteins in fission yeast meiosis: We assume that meiotic recombination in *S. pombe* is largely initiated by DSBs. On the basis of our findings we propose a model for contribution of *S. pombe* RecA-like proteins to different meiotic events (Figure 2). Our observations suggest that Rad51 and Dmc1 are key functions required for meiotic recombination and essentially no recombination occurs in their combined absence. There is quite a high level of recombination in the absence of only Dmc1 and a lower, but still significant level in the absence of only Rad51 (Figure 1). Therefore, we distinguish two pathways: the Rad51 pathway promotes recombination in the *dmc1* null mutant, and the Dmc1 pathway promotes recombination in the *rad51* null mutant. The respective involvement of these pathways in crossovers and conversions and in spore viability is indicated in Figure 2 as open and solid bars, respectively. The corresponding values for the Rad51 pathway derive from the *dmc1* mutant and those for the Dmc1 pathway derive from the *rad51* mutant (Figure 1). When both proteins are present in the cell, there is more recombination than the sum of the events promoted by the isolated Rad51 and Dmc1 pathways (*dmc1* and *rad51* mutants). The values obtained for wild type were set to 100%. The additional events occurring in the presence of both proteins were calculated as 100% minus the values for *rad51* and *dmc1* mutants and tentatively assigned to a third pathway (Figure 2, shaded bars). Accordingly, the corresponding values for *rhp55*$^-$ *rhp57*$^-$ background were determined, but expressed in percentage of the wild-type strain (not in percentage of the values obtained in the *rhp55 rhp57*$^-$ double mutant). The analysis of strains carrying deletions of *rhp55* and/or *rhp57* revealed that Rhp55 and

Rhp57 act in the same pathway with Rad51 and stimulate its activity (Figure 2, pointed arrows). Rhp57 (and probably Rhp55) partially inhibit intragenic recombination carried out by the Dmc1 pathway (Figure 2, blunt arrow). With respect to different meiotic events Rlp1 can be assigned to either Rad51 or Dmc1 pathways or both. The data are not sufficient for a clear description of its role in the Rad51 and/or Dmc1 pathways. The only evident conclusion is that intragenic recombination is lower in the presence of Rlp1 than in its absence. We therefore indicate an inhibitory role of Rlp1 in both the Rad51 and Dmc1 pathways with respect to gene conversion (Figure 2, blunt arrows).

The levels of spore viability and crossovers provided by the Dmc1 pathway alone do not change significantly in the absence of *rhp55*$^+$ and *rhp57*$^+$, indicating that these functions of Dmc1 are not influenced by *rhp55*$^+$ and *rhp57*$^+$. However, gene conversion performed by the Dmc1 pathway is more efficient in the absence of *rhp55*$^+$ and *rhp57*$^+$ than in their presence, indicating an inhibitory effect of these proteins on Dmc1 activity.

The levels of spore viability and gene conversion provided by the Rad51 pathway alone are significantly lower in the absence of *rhp55*$^+$ and *rhp57*$^+$, indicating that Rhp55 and Rhp57 stimulate these activities of Rad51. The number of Rad51-dependent crossovers does not change significantly in the absence of *rhp55*$^+$ and *rhp57*$^+$. This indicates that some activities of Rad51 are strongly dependent on Rhp55 and Rhp57, while others are not.

The recombinogenic activity of the third tentative pathway (Rad51 and/or Dmc1) is reduced in the absence of *rhp55*$^+$ and *rhp57*$^+$. In contrast, the viability of spores is increased. We propose that some of the functions of this pathway are assisted by Rhp55 and Rhp57, while others are inhibited.

For the third tentative pathway the possibility exists that Rad51 is needed for all recombination events, while Dmc1 does not perform recombination when Rad51 is absent. Instead, it would be required to direct Rad51 toward interhomolog recombination rather than intersister recombination. The budding yeast Dmc1 is proposed to be involved in the establishment of such a bias in *S. cerevisiae* (Schwacha and Kleckner 1997). Our data are consistent with a similar role of Dmc1 in *S. pombe*: we detect more interhomolog recombination events (in our system only interhomologous exchanges are detectable) in the presence of Rad51 together with Dmc1 than in the presence of Rad51 alone.

What are the different pathways and why do they involve different RecA-like proteins? In analogy to the scheme of cellular intermediary metabolism, which has evolved from "one gene-one enzyme" linear pathways to a network of pathways (with branches, more than one way to get from a substrate to a product, and enzymes recognizing several different substrates), we assume that during meiotic recombination DSBs can be repaired via different recombination intermediates leading to different products. If in such a network every node is considered an intermediate, and the arrows between the nodes are the reactions carried out by one or more enzymes, then any tract of arrows connecting a lesion (substrate) through several nodes (intermediates) to a product can be considered a pathway. RecA-like proteins may have different affinities to DNA intermediates varying in some structural aspects and catalyze their transformation to one or the other product (depending on cofactors). In addition, inhibition of certain reactions may also be an activity of specific RecA-like proteins. In Figure 2 we began to draw such a network of recombination pathways for *S. pombe* meiosis. In conclusion, the presented synoptic analysis of the roles of the five *S. pombe* RecA-like proteins in meiotic recombination and spore viability forms a basis for the more detailed study of the specific functions of the individual proteins.

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